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**TITLE: Examination of Neuroimaging, Cognitive Functioning, and Plasma Markers in a Longitudinal Cohort of Gulf War Deployed Veterans: The Ft Devens Cohort**

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# REPORT DOCUMENTATION PAGE

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<b>14. ABSTRACT</b> This study aims to conduct follow-up longitudinal cognitive evaluations on a sub-sample of 100 Time 3 Ft. Devens Cohort veterans, most of whom were last evaluated in-person for cognitive functioning and with neuroimaging in the mid-1990s, to compare objective measurement of reported decline; and to determine cross-sectional blood and neuroimaging biomarkers (blood and structural volumetrics will also include longitudinal analyses) at 25+ years post deployment to the Gulf region, that may be consistent with cognitive outcomes and presumed pathobiological mechanisms (oxidative stress, ROS) of GWI. These data will evaluate the utility of previously unavailable blood and neuroimaging markers of oxidative stress, to devise a new diagnostic test for GWI in subgroups of GW veterans (TBI and OP exposed), and to provide a potential objective biomarker of treatment efficacy in clinical trials.				
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## **1. Introduction:**

**Background and Purpose:** One of the earliest and longest running studies of Gulf War veteran's (GWV) health was conducted with the Ft. Devens, MA army cohort (FDC) from the VA Boston Healthcare System (VABHS). The cohort was first surveyed within 5 days of their return and multiple cross sectional survey and in-person data provided some of the earliest cognitive, neuroimaging and environmental exposure outcomes since the 1990s. These findings included early documentation of the most common health symptoms, cognitive decrements in environmentally exposed GWV, and differences in structural neuroimaging, including lower white matter volumes (Proctor et al., 1998; White et al., 2001; Heaton et al., 2007). The FDC has been followed with longitudinal health surveys, and we are completing a resurvey and biomarker study in which 50% of prior surveyed individuals have responded. This most recent resurvey is providing valuable information pertaining to longitudinal health symptoms and the trajectory of health symptoms over time. Additionally, we are able to use this longitudinal self-report data to monitor CDC and Kansas GWI criteria over time. However, these data are self-report and only a small portion of individuals are being seen for cross-sectional analyses of proteins in the blood (GW100046). Since subsamples of the FDC took part in cognitive and neuroimaging studies between 1994 and 1996, we are now extending these studies by reassessing neurocognitive and neuroimaging status to more fully capitalize on the longitudinal nature of this cohort and the recent findings of oxidative stress markers in GWI.

**Scope:** The overarching objective of this work study is to build on previous longitudinal studies to gain a better understanding of Gulf War Illness and to devise targeted treatment strategies. This study aims to conduct follow-up longitudinal cognitive evaluations on a sub-sample of 100 Time 3 FDC veterans, most of whom were last evaluated in-person for cognitive functioning and with neuroimaging in the mid-1990s, to compare objective measurement of reported decline; and to determine cross-sectional blood and neuroimaging biomarkers (blood and structural volumetrics will also include longitudinal analyses) at 25+ years post deployment to the Gulf region, that may be consistent with cognitive outcomes and presumed pathobiological mechanisms (oxidative stress, ROS) of GWI. These data will evaluate the utility of previously unavailable blood and neuroimaging markers of oxidative stress, to devise a new diagnostic test for GWI in subgroups of GWV (TBI and OP exposed), and to provide a potential objective biomarker of treatment efficacy in clinical trials.

**2. Key Words:** Gulf War Illness, central nervous system, biomarkers, glutathione, MR Spectroscopy, Cognition, oxidative stress

## **3. Accomplishments:**

- What were the major goals of the project?
  - The major goals of the project as stated in the approved SOW for year1 is listed in the table below. Specifically, during year 1, the primary goals were to obtain Boston VA and Boston University IRB approvals and DOD HRPO approvals. Planning for study protocols for brain imaging, cognitive testing and oxidative stress blood markers was also a goal of this year. Milestones/target dates for important activities or phases of these dates are listed in the table and actual completion dates are listed below.

<b>Tasks</b>	<b>Timeline</b>
<b>Task 1. Obtain necessary authorization prior to initiation of human subjects</b>	<b>Months</b>
1a. Obtain Institutional Review Board (IRB) approval for research sites at VA Boston (VABHS), Boston University Medical Campus (BUMC), and Nova University (NSU) for protocols	1-4
1b. Obtain DOD Human subjects Research Protections Office (HRPO) approvals	5-7
1c. Complete hiring of necessary staff and ensure all mandatory IRB research related trainings are completed by all staff members	1-8
<b>Task 2. Preparation and Training for Clinical Study Procedures</b>	<b>Months</b>
2a. Obtain Time 3 cognitive and MRI neuroimaging data for longitudinal analyses and participant contact information from the Ft. Devens cohort (FDC) study through the share drive at VABHS.	1-2
2b. Develop manuals for neuropsychological testing protocol, structural MRI and Magnetic Resonance Spectroscopy (MRS) of glutathione oxidative stress marker (GSH) protocols and blood specimen collection protocols for several oxidative stress markers.	1-6
2c. Train researchers and staff on cognitive, neuroimaging and phlebotomy protocols and quality control measures.	6-9
<b>Task 3. Screening, recruitment and longitudinal assessment of FDC Gulf War veterans</b>	<b>Months</b>
3a. Obtain informed consent from potentially eligible GW veterans	9-36
3b. Assess 150 FDC veterans and obtain demographics, medical history, self-report questionnaires and neuropsychological testing for planned longitudinal analyses.	9-36
3c. Perform brain GSH MR Spectroscopy and structural MRI imaging and blood draw for oxidative stress markers from 100 Gulf War veterans for cross-sectional study.	9-36
<b>Task. 4. Data Cleaning and MRI/MRS image Post-processing</b>	<b>Months</b>
4a. Post-process MRI/MRS neuroimaging data for data analysis.	18-40
4b. Score neuropsychological test data and upload summary data to VA Share drive for entry, cleaning and analyses.	18-38
4c. Ship blood samples to Nova University for analysis of GSH oxidative stress markers including (HNE, 8-iso-PGF2α).	18-36
4d. Perform analyses of plasma oxidative stress markers.	18-40
<b>Task. 5. Merge Data and Perform Interim Data analyses</b>	<b>Months</b>
5a. Data entry of all questionnaires, cognitive evaluations and quality control measures will be ongoing.	18-42
5b. Interim Statistical analyses of data obtained from cognitive evaluations, blood markers, neuroimaging and questionnaire data will	18-42

be performed periodically.	
5c. Annual reports of progress will be written.	18-36
<b>Task 6. Perform Final Data Analysis and Prepare Manuscripts for Publication (months 42-48)</b>	<b>Months</b>
6a. Perform cross-sectional analyses comparing central and peripheral markers of oxidative stress in brain MRS (GSH) and plasma (HNE, 8-iso-PGF2 $\alpha$ ) compared with cognitive functioning and health symptom report in FDC veterans.	42-45
6b. Perform longitudinal analyses of structural MRI imaging, cognitive, and health symptom outcomes from Time 3 and Time 6 in FDC veterans.	42-46
6c. Write final study report	47-48
6d. Present findings at scientific meetings	42-48
6e. Prepare manuscripts for submission for cross-sectional and longitudinal studies.	42-48

- What was accomplished under these goals?
- Task 1:
  - We obtained necessary authorization prior to initiation of study (IRB approvals, DoD HRPO approvals).
  - We completed the hiring of staff and ensured that all mandatory trainings are now completed.
- Task 2:
  - We have obtained Time 3 cognitive and MRI neuroimaging data for the longitudinal analysis and participant contact information form the Ft. Devens cohort (FDC) study and Treatment Seeking Cohort (TSC) through the share drive at VA Boston Health Care System (VABHS).
  - We developed manuals for the neuropsychological testing protocol as well as structural MRI and MRS of glutathione oxidative stress marker (GSH) protocols and blood specimen collection protocols for oxidative stress markers.
  - We have trained the researchers and staff on cognitive neuroimaging and phlebotomy protocols and questionnaires.
- How were the results disseminated to communities of interest?
  - Our results from analyzing data from the Ft. Devens cohort specifically related to mTBI were presented at two meetings of the International Neuropsychological Society meetings and two recently published papers (see Appendix).
- What do you plan to do during the next reporting period to accomplish the goals?
  - We plan to recruit 50 study participants by the next reporting period and present the preliminary results at appropriate National and International meetings.
  - We plan to submit other manuscripts of preliminary results from the GWI case-control and mTBI exposed vs non-exposed groups and the cognitive, MR Spectroscopy glutathione brain imaging and blood oxidative stress markers during the next reporting period.

#### **4. Impact:**

- What was the impact on the development of the principal discipline(s) of the project?
  - Gulf War Illness (GWI) can have a dramatic impact on the lives and well-being of GW veterans who experience chronic and often debilitating symptoms. The results of this study will help address a critical knowledge gap regarding the nature of continued cognitive symptoms and other chronic health effects of GWI.
  - This project will distinguish itself by examining the nature and trajectory of symptoms by adding objective markers of longitudinal neurocognitive decline, traditional structural MRI imaging and cutting-edge MRS brain imaging techniques of oxidative stress markers (glutathione) compared with plasma oxidative stress markers. When combined with the prior rich 20+ year longitudinal data from the Ft. Devens and Treatment-seeking cohorts, this provides an unprecedented opportunity to further characterize objective biomarkers of illness in a well-characterized cohort of GW veterans.
  - Defects in modulation of oxidative stress may well predispose individuals to damage from reactive oxygen species (ROS) from environmental exposures, TBI or other sources that could potentially be used as a diagnostic marker of illness.
  - This analysis also offers an opportunity to determine whether a given therapeutic strategy such as antioxidants including co-enzyme Q-10 or quercetin supplementation in subgroups with low brain glutathione levels may be chosen as a treatment option to improve a susceptible individual's ability to modulate oxidative stress, reduce accelerated aging and improve the symptoms of GWI utilizing a personalized medicine approach.
- What was the impact on other disciplines?
  - A major advantage of work with the Ft. Devens cohort showing mTBI to be related to rates of GWI in our two most recent papers suggests that the results of this study with oxidative stress glutathione markers may be relevant not only to GWI but also to other veteran and civilian groups with mTBI and neurotoxicant exposures as part of a multiple-hit hypothesis.
  - Blood and neuroimaging-based biomarkers of GWI provide an effective way to enhance its management:
    - It can be used as a diagnostic and prognostic tool with the ability to provide information about rate of disease progression.
    - It would help in identification of novel and effective treatments for multiple disorders and environmental exposure groups (i.e. pesticides, nerve agents).
    - It could be used for monitoring therapeutic efficacy for multiple disorders
    - It could provide a cost-effective option for recruitment into clinical trials
- What was the impact on technology transfer?
  - The biomarker that we hope to develop will be cost effective, available, and do not need expensive technicians if we can identify an oxidative stress biomarker in blood that be correlated with MR spectroscopy brain imaging markers that we will also collect and analyze.

- What was the impact on society beyond science and technology?
  - Our blood and brain imaging biomarkers should improve the quality of life for the veterans of the GW who have GW illness because:
    - Our biomarkers can provide objective evidence thereby validating the chronic health symptoms of ill GW veterans.
    - Our biomarkers should lead to studies to develop treatment of brain injury that may lead to improvement of their clinical condition.

## **5. Changes for approach and reasons for change:**

- Changes:
  - None
- Problems:
  - No problems
- Actual or anticipated problems or delays and actions or plans to resolve them
  - We have been slightly delayed in getting subject recruitment started but we recently had a team meeting in Boston to finalize our plans for recruitment and our study coordinator began contacting FDC veterans to start subject recruitment for the study.
- Changes that had significant impact on expenditures
  - None
- Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents:
  - Significant changes in use or care of human subjects: None
  - Significant changes in use or care of vertebrate animals: None
  - Significant changes in use of biohazards, and/or select agents: None

## **6. Products:**

- **Publications, conference papers, and presentations**
  - **Journal Publications**
- Yee MK, Janulewicz PA, Seichepine DR, Sullivan KA, Proctor SP, Krengel MH. Multiple Mild Traumatic Brain Injuries Are Associated with Increased Rates of Health Symptoms and Gulf War Illness in a Cohort of 1990-1991 Gulf War Veterans. *Brain Sci.* 2017 Jul 9;7(7). pii: E79. doi: 10.3390/brainsci7070079. See Appendix 1.
- Yee, M., Seichepine, D., Januelwicz Lloyd P., Sullivan, K., Proctor, SP & Krengel, M. Traumatic brain injury, health and rate of chronic multisymptom illness in veterans from the 1990-1991 Gulf War. *Journal of Head Trauma Rehabilitation.* 2016 Sep-Oct;31(5):320-8. doi: 10.1097/HTR.0000000000000173.
- **Books or other non-periodicals, one-time publications**
  - None

- **Other publications, conference papers, and presentations**
  - Two posters were presented during the annual International Neuropsychological Society Meeting in Washington, DC entitled:
  - Krengel MH, Yee M, Nolan T, Janulewicz Lloyd PA, Sullivan K & Seichepine DR. Multiple Self-Reported Exposures to Mild Traumatic Brain Injury and Neurotoxicants Predict Current Total Health Symptoms in a Cohort of 1990-1991 Gulf War Veterans. Journal of International Neuropsychological Society. Supplement 1, March 2016.
  - Yee, M., Seichepine DR, Nolan T, Janulewicz Lloyd PA, Sullivan K & Krengel MH. Multiple Self-Reported Brain Injuries are Associated with Increased Health Symptoms in a Cohort of 1990-1991 Gulf War Veterans. Journal of International Neuropsychological Society. Supplement 1, March 2016.
- **Other products**
- **Website(s) or other Internet site(s)**
  - None
- **Technologies or techniques**
  - None
- **Other products**
  - None

## **7. Participants and other Participating Organizations**

Site 1: VA Boston Health Care System (VABHS)  
(BUMC) 150 S. Huntington Avenue  
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Initiating PI: Maxine Krengel, PhD

Site 3: Nova Southeastern University (NSU)  
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Fort Lauderdale, Florida 33328-2018  
Co-Investigator: Richard C. Deth, PhD  
Collaborator: Nancy Klimas, MD

Site 2: Boston University Medical Campus  
715 Albany Street  
Boston, MA 02118  
Partnering PI: Kimberly Sullivan, PhD  
Co-Investigator: Carole Palumbo, PhD  
Co-Investigator: Ronald Killiany, PhD

### **Study Sites Responsibilities**

**Site 1:** Dr. Krengel and her VABHS team will be responsible for recruiting FDC study participants and conducting cognitive evaluations and phlebotomy to send to NOVA investigators. Specifically, she will oversee the recruitment and blood draws/cognitive evaluations of FDC study participants and the processing of plasma samples that will be shared for the proposed study. Dr. Krengel will also oversee the experimental design, data analysis and

interpretation and presentation of study results in collaboration with Dr. Sullivan and the other study investigators. **Tasks 1-6**

**Site 2:** Dr. Sullivan and her BUMC team will be responsible for performing the MRS/MRI imaging protocols and post-processing the imaging data for cross-sectional (MRS) and longitudinal analyses (structural MRI). Specifically, she will oversee the imaging acquisition and post-processing protocols in collaboration with Drs. Killiany and Palumbo. Dr. Sullivan will also assist with the experimental design, data analysis and interpretation and presentation of study results in collaboration with Dr. Krengel and the other study investigators. **Tasks 1-6**

**Site 3:** Dr. Deth and Klimas will be responsible for receiving the plasma samples from the Boston site and performing oxidative stress marker analyses for GSH, HNE and 8-iso-PGF $\alpha$  for 100 blood samples (50 GWI, 50 controls). Dr. Deth will also assist with the experimental design, interpretation of data, report and manuscript writing and presentation of results at scientific meetings. **Tasks 1, 2, 4, 5, 6**

Reporting Requirements:

None

# Self-Reported Traumatic Brain Injury, Health and Rate of Chronic Multisymptom Illness in Veterans From the 1990-1991 Gulf War

Megan K. Yee, MA; Daniel R. Seichepine, PhD; Patricia A. Janulewicz, DSc;  
Kimberly A. Sullivan, PhD; Susan P. Proctor, DSc; Maxine H. Krengel, PhD

**Background:** Traumatic brain injury (TBI) was not considered to be common in the 1990-1991 Gulf War (GW). Therefore, the relationship between TBI and chronic health symptoms experienced by GW veterans is unknown. Health symptoms reported by veterans deployed more recently to this region (Operations Enduring and Iraqi Freedom) are similar to those of GW veterans and have been primarily attributed to TBI. **Objective:** To examine the relationships among self-reported TBI, health symptoms, chronic multisymptom illness (CMI), and health-related quality of life among GW veterans. **Participants:** Participants included 1 274 GW veterans from the Devens Cohort Study, 156 of whom self-reported a history of TBI (12.2% of the sample). **Design:** Cross-sectional retrospective analysis of existing survey data. **Main Measures:** A 52-item health symptom checklist and the RAND 36-Item Health short Form Survey. **Results:** Self-reported TBI in GW Veterans is related to increased rates of health symptoms, CMI, and poorer health-related quality of life. **Conclusions:** Gulf War veterans' self-reported exposure to TBI is related to increased rates of chronic health symptoms and CMI, which interfere with everyday activities of daily living. **Key words:** *chronic multisymptom illness, Gulf War, traumatic brain injury*

OVER THE PAST 3 DECADES, veterans from the 1990-1991 Gulf War (GW) have consistently reported numerous chronic health symptoms affecting

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**Author contributions:** Megan K. Yee was responsible for data analysis, drafting the manuscript, and interpretation of findings. She reports no disclosures. Daniel R. Seichepine was responsible for data analysis, drafting the manuscript, and interpretation of findings. He reports no disclosures. Patricia A. Janulewicz was responsible for contributing to the interpretation of findings. She reports no disclosures. Kimberly A. Sullivan was responsible for contributing to the interpretation of findings. She reports no disclosures. Susan P. Proctor has been the past Director of the Devens Cohort Study. For this manuscript, she was responsible for review and editing of the manuscript, including particular attention to consistent reporting of data metrics and historical aspects pertaining to the Devens Cohort Study. She reports no disclosures. Maxine H. Krengel was responsible for contributing to the interpretation of findings. She reports no disclosures.

The views expressed in this article are those of the authors and do not reflect the official policy or position of the Department of the Army or Department of Veterans Affairs.

The authors report no conflicts of interests.

their quality of life.<sup>1-14</sup> Despite considerable investment directed at discovering the etiologies of these chronic health symptoms among GW veterans, the cause or causes remain elusive.<sup>15</sup> In the more recent wars in this region (Operations Enduring Freedom and Iraqi Freedom [OEF/OIF]), veterans have also reported some similar chronic health symptoms, which have been primarily attributed to mild traumatic brain injuries (mTBI).<sup>16-19</sup> However, the relationship between TBI and chronic health symptoms in veterans from the 1990-1991 GW has not been studied. Due to the limited ground war, TBI was not considered a risk factor for chronic multisymptom illness because these types of injuries were thought to be infrequent or nonexistent during the GW.

The constellation of symptoms associated with GW deployment has been termed by the Centers for Disease Control and Prevention as chronic multisymptom illness<sup>20</sup> (CMI; also referred to as Gulf War Illness, Gulf War Syndrome, or Medically Unexplained Symptoms).<sup>21</sup> Some 12% to 66% of GW veterans have

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endorsed symptoms consistent with CMI.<sup>15</sup> The etiology of CMI has been controversial because of inconsistent and varied results. Theories have speculated on the impact of stress, prophylactic treatments (eg, vaccinations and anti-nerve gas pills), and environmental contaminants (eg, sarin/cyclosarin, pesticides).<sup>15,22</sup>

In general, military personnel have increased rates of TBIs when compared with the civilian population.<sup>23</sup> Since the Department of Defense began documenting TBIs in 2000, nearly 300 000 cases have been reported. Battle-related TBIs only account for a minority of these injuries (~11%) with the most common causes including motor vehicle accidents (about 20%), falls (about 20%), and being struck by or against objects (15%).<sup>24</sup> The majority of these injuries are classified as mild and occur when a force to the head or neck results in the transient disruption of brain functioning causing loss of consciousness for less than 30 minutes, alteration of mental state for less than 24 hours, and posttraumatic amnesia for less than 1 day.<sup>25</sup> Mild TBIs often go unrecognized when the acute symptoms are of short duration (eg, a few seconds), are underreported, or are attributed to other causes by the patient or clinician<sup>26</sup>; as such mTBI has been described as a “silent epidemic.”<sup>27</sup>

In response to growing concerns about the effects of unrecognized mTBIs in OEF and OIF veterans, the Department of Veterans Affairs mandated a standardized screening and comprehensive evaluation process for all returning veterans starting in 2007. Based on this postdeployment evaluation, more than 30 000 veterans have received a clinical diagnosis of TBI, but more than 44 000 reported moderate-to-severe postconcussive symptoms.<sup>28</sup> Those with TBI also report chronic symptoms, some of which are similar to those experienced by GW-deployed veterans including neck aches, backaches, irritability, fatigue, and nausea. The extent to which these subjective self-reported health symptoms indicate an undiagnosed brain injury is unclear. However, military personnel training to safely use explosives have shown an increase in an objective biomarker of brain injury in addition to reporting an increase in postconcussive symptoms, despite not being diagnosed with TBI.<sup>29</sup> Similar findings have also been reported in various nonmilitary cohorts.<sup>30-33</sup> Taken together, these findings suggest that although postconcussive symptoms are recognized by the patient and may be correlated with an objective biomarker of brain injury, they do not always lead to a clinician-diagnosed TBI.

The degree to which TBI contributes to chronic health symptoms reported by veterans from the 1990-1991 GW is unknown. Given that the etiology of these symptoms remains unclear, and that TBI has been linked to similar postdeployment symptoms in other veteran populations, this study aimed at examining the cross-sectional relationships among self-reported TBI and health symp-

toms, CMI, and health-related quality of life in GW veterans. We hypothesized that GW veterans with reported TBI will endorse significantly higher rates of health symptoms and CMI as well as worse health-related quality of life.

## METHOD

### Participants

Participants included 1 274 GW veterans from the Devens Cohort Study, which is composed of approximately 100 US Army units that returned from the war in 1991 through Fort Devens in Massachusetts. Approximately 92% of the participants reported being enlisted at the time of the GW, and the remaining 8% reported being officers. The Devens Cohort Study was the first research investigation of GW veterans following their return from deployment.<sup>34</sup> The initial purpose of the study was to assess psychological readjustment post-deployment. Later assessments of the cohort included both physical and emotional health concerns. The cohort completed postdeployment questionnaires at the following 3 time points: 1991, 1992 to 1993, and 1997 to 1998. In addition, a stratified random subset of the cohort was assessed in-person in 1994 to 1996.<sup>3,5,7,8,10</sup> Cross-sectional data from the 1997 to 1998 survey were analyzed for this study.<sup>3-10</sup> To determine TBI history, participants reported “yes” or “no” if they have ever had a “head injury, concussion, or period of being knocked unconscious.” One hundred fifty-six participants self-reported a history of TBI (12.2% of the sample; TBI group) and 1 118 denied a TBI history (no-TBI group). The groups were similar in age, education, race, and ethnicity (see Table 1).

### Health symptom checklist

The health symptom checklist (HSC) is a 52-item self-report questionnaire assessing the presence or absence of bothersome health symptoms over the past 4 weeks. Participants were instructed to check “yes” if the symptom was present during that time frame and “no” if it had been absent. If present, the participants indicated whether the symptom was bothersome either “sometimes” or “a lot.” The focus of this study was the presence or absence of symptoms over the past 4 weeks.

### CMI criteria

Centers for Disease Control and Prevention criteria for CMI include the presence of persistent health symptoms for 6 months or longer in 2 of the 3 following categories: fatigue, musculoskeletal factors, and mood and/or cognition. Participants self-reported the presence or absence of symptoms in each of these domains over

**TABLE 1** Demographic characteristics

	No TBI (n = 1 118)	TBI (n = 156)
Age, <sup>a</sup> y	38.1 (9.0)	37.5 (8.4)
Education, <sup>a</sup> y	13.7 (1.9)	14.0 (1.8)
Male:female <sup>b</sup>	1 010:105	138:16
Race/ethnicity <sup>c</sup>		
Black or African American	5.1%	1.9%
White	90.3%	94.8%
Hispanic	2.6%	1.9%
Asian/Pacific Islander	0.6%	0.6%
American Indian/Alaska Native	0.8%	0.0%
Other/Unknown	1.0%	1.9%
Occupation in military		
Military police	31.6%	34.6%
Medical	15.8%	14.1%
Transportation	15.2%	15.4%
Engineering	14.0%	10.3%
Special forces	11.8%	16.7%
Other/unknown	11.6%	8.9%

<sup>a</sup>Means and standard deviations reported for age and education.

<sup>b</sup>Sex was not reported by 5 participants.

<sup>c</sup>Race rounded to the nearest one tenth of a percentage point.

the past 6 months and were classified accordingly as exhibiting CMI or not.<sup>20</sup>

### RAND 36-Item Short Form Health Survey 1.0

The RAND 36-Item Short Form Health Survey 1.0 (SF-36) is a 36-item self-report questionnaire used to evaluate health-related quality of life. This commonly used measure has well-established reliability and validity for a diverse range of populations including persons with TBI.<sup>35-38</sup> Higher scores represent better functioning. Responses load onto 2 component scores, Physical Component Summary (PCS) and Mental Component Summary (MCS); each comprises 4 individual subscales. The PCS includes these subscales—(1) Physical Functioning, which evaluates limitations in performing physical activities; (2) Role-Physical, which evaluates limitations with work or other daily activities due to physical health; (3) Bodily Pain, which evaluates limitations with work or other daily activities due to pain; and (4) General Health, which evaluates the respondents' perception of overall health. The MCS includes the following subscales: (1) Vitality, which evaluates feeling tired or worn out; (2) Social Functioning, which concerns problems with social activities due to physical and emotional problems; (3) Role-Emotional, which evaluates problems with work or other activities due to emotional problems; and (4) Mental Health, which addresses feelings of nervousness

or depression. Scores were calculated using the method described by Ware et al.<sup>39</sup>

### Statistical analysis

#### Analysis of HSC

The chi-square test of independence was used to compare the rates of each of the 52 health symptoms in the TBI and no-TBI groups. To control for type I errors, alpha level was conservatively adjusted to 0.001 (0.05/52 = 0.001) using the Bonferroni method.<sup>40,41</sup> Eight participants in the no-TBI group, who completed less than 80% of the HSC, were excluded from the analysis.

#### Analysis of CMI

The chi-square test of independence was used to compare the rate of CMI between the TBI and no-TBI groups. An alpha level of 0.05 was adopted. The CMI status was available for all participants in the TBI group, but not for 7 participants in the no-TBI group, and therefore they were excluded from the analysis.

#### Analysis of SF-36

Because of the nonnormal distribution of the data, between-group comparisons were performed using the nonparametric Mann-Whitney *U* test. For the PCS and MCS, an alpha level of 0.05 was adopted. For follow-up analyses of the 4 individual subscales, which make up each component score, alpha was adjusted to 0.0125 (0.05/4 = 0.0125). Fifteen of the 1 274 participants (1.2%; 1 in the TBI group; 14 in the no-TBI group) had at least 1 missing data point on the SF-36 and therefore were excluded from the analysis.

## RESULTS

### Comparison between the TBI and no-TBI groups on the HSC

Rates of the following 8 health symptoms were significantly higher in the TBI group when compared with the no-TBI group—racing heart, nausea or upset stomach, muscle weakness or fatigue, neck aches or stiffness, backaches, thickened saliva, fatigue or easily tired, and excessive anger or irritability (all  $P \leq .001$ ) (see Table 2).

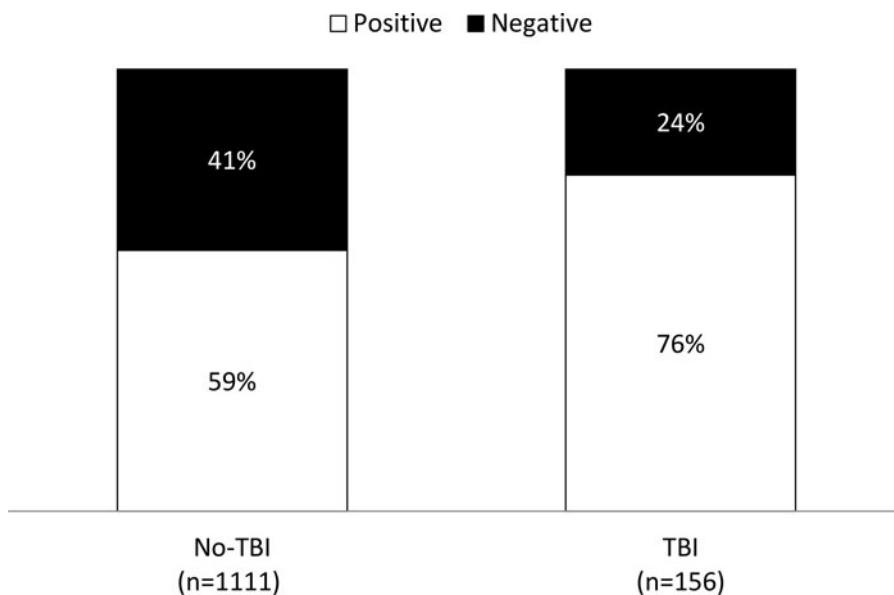
### Comparison between TBI and no-TBI groups on rate of CMI

Rate of CMI in the TBI group (76%) was significantly higher than in the no-TBI group (59%;  $P < .000$ ) (see Figure 1).

**TABLE 2** Rates of health symptoms in Gulf War veterans with and without TBI

<b>Health Symptom</b>	<b>No TBI</b>	<b>TBI</b>	<b>P</b>
Cardiac system			
Racing heart	13.7%	23.9%	.001 <sup>a</sup>
Irregular heart beat or heart flutters	12.5%	19.2%	.021
Chest pain	20.4%	28.8%	.016
Dermatological system			
Skin rash, eczema, or skin allergy	29.7%	39.1%	.017
Gastrointestinal system			
Nausea and/or upset stomach	27.2%	44.2%	.000 <sup>a</sup>
Stomach cramps or excessive gas	37.9%	47.4%	.022
Diarrhea or constipation	31.5%	41.6%	.013
Genitourinary system			
Frequent urination	18.9%	24.0%	.142
Pain during intercourse	5.0%	9.7%	.020
Difficulty achieving orgasm	11.0%	18.1%	.011
Musculoskeletal system			
Muscle weakness or fatigue	45.0%	60.0%	.000 <sup>a</sup>
Muscle twitching or shakiness	30.2%	38.6%	.036
Neck aches or stiffness	37.6%	53.2%	.000 <sup>a</sup>
Backaches	44.2%	60.1%	.000 <sup>a</sup>
Joint pains	53.9%	65.4%	.007
Neurological system			
Headaches	39.8%	52.9%	.002
Dizziness or feeling lightheaded	27.2%	37.2%	.010
Ringing in ears	29.5%	39.7%	.009
Blurred vision	22.5%	27.1%	.211
Difficulty swallowing	9.3%	16.1%	.008
Numbness in arms or legs	29.3%	33.1%	.328
Hallucinations	2.5%	3.3%	.602
Dry mouth	16.4%	26.0%	.004
Excess sweating or perspiration	18.8%	23.9%	.132
Hands feeling sweaty, damp, or clammy	11.6%	16.7%	.067
Feeling hot or cold for no reason	20.1%	21.8%	.616
Thickened saliva	6.9%	15.6%	.000 <sup>a</sup>
Neuropsychological system			
Forgetfulness	53.7%	63.2%	.026
Difficulty learning new material	18.1%	18.8%	.084
Blank spots in memory	35.1%	41.3%	.131
Confusion	18.9%	25.8%	.042
Difficulty concentrating	34.2%	46.2%	.004
Psychological system			
Feeling generally ill	21.6%	26.9%	.133
Unable to fall asleep	35.1%	47.4%	.003
Restless, unsatisfying sleep	50.0%	63.6%	.002
Awake earlier than desired	37.2%	48.1%	.009
Fatigue or easily tired	52.3%	67.7%	.000 <sup>a</sup>
Taking meds to sleep or calm down	12.0%	14.1%	.452
Nail biting	23.1%	26.3%	.387
Restless or cannot sit still	27.8%	34.2%	.100
Frequent anxiety or nervousness	29.4%	38.7%	.018
Excessive anger or irritability	42.5%	61.7%	.000 <sup>a</sup>
Frequently feeling depressed	32.1%	38.5%	.111
Crying easily	15.1%	21.9%	.032
Diminished or no appetite	10.4%	16.9%	.016
Excessive hunger or eating	18.5%	25.2%	.049
Feeling that life is pointless	15.3%	21.2%	.061
Loss of interest in TV, movies, news, or friends	17.5%	23.9%	.055
Loss of interest in sex	23.4%	32.5%	.014
Pulmonary system			
Rapid breathing	11.4%	18.8%	.009
Difficulty breathing or shortness of breath	28.9%	34.0%	.195
Common cold or flu	34.6%	42.9%	.041

<sup>a</sup>P < .001.



**Figure 1.** Rate of CMI for the TBI group and no-TBI group. The TBI group had higher rate of CMI when compared with the no-TBI group. CMI, chronic multisymptom illness; TBI, traumatic brain injury.

### Comparison between TBI and no-TBI groups on the SF-36

The TBI group reported significantly worse functioning for the PCS compared with the no-TBI group ( $TBI = 47.7 \pm 10.06$ ; no-TBI =  $44.7 \pm 11.18$ ;  $P = .000$ ), but not for the MCS ( $TBI = 45.3 \pm 12.0$ ; no-TBI =  $43.5 \pm 12.8$ ;  $P = .143$ ). Significant between-group differences were found on 3 of the 4 individual subscales that make up the PCS. Relative to the no-TBI group, the TBI group reported worse functioning for Physical Functioning ( $P = .001$ ), Bodily Pain ( $P = .001$ ), and General Health ( $P = .001$ ) individual subscales. Groups were similar on the Role-Physical individual subscale ( $P = .015$ ). Significant between-group differences were found on 1 of the individual subscales that make up the MCS. Relative to the no-TBI group, the TBI group reported worse symptoms on Vitality ( $P = .009$ ), but not Social Functioning ( $P = .025$ ), Role-Emotional ( $P = .118$ ), or Mental Health ( $P = .056$ ) (see Figures 2 and 3).

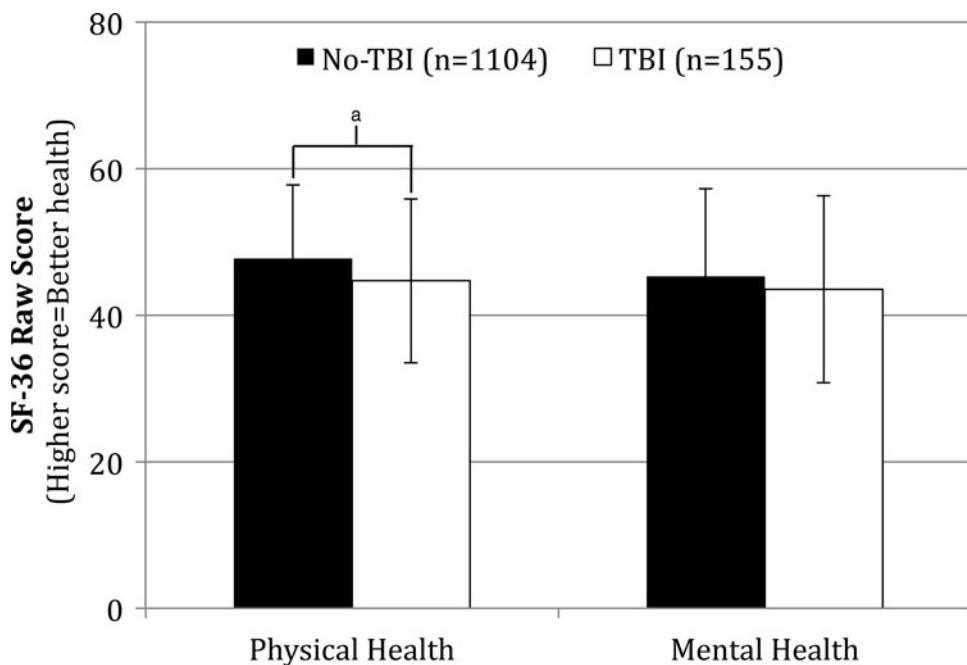
### DISCUSSION

This study examined the relationships among self-reported TBI and health symptoms, CMI, and health-related quality of life in a cohort of 1990-1991 GW veterans. Traumatic brain injury has not previously been considered a contributing factor to the chronic health symptoms experienced by GW veterans, but it has been connected to poorer health in OEF/OIF veterans and civilian populations.<sup>16-19,42-44</sup> Overall, we found that GW veterans with a self-reported history of TBI report higher rates of health symptoms and are more likely to meet criteria for CMI when compared with veterans

without a self-reported history of TBI. These symptoms are not inconsequential, as results from the SF-36 indicate that they appear to interfere with everyday activities of daily living, including performing household chores, working, and leisure activities.

Consistent with our hypotheses, rates of self-reported health symptoms (as measured by HSC) were higher overall for GW veterans with a self-reported history of TBI, with 8 specific symptoms spanning 5 body systems (ie, cardiac, gastrointestinal, musculoskeletal, neurological, and psychological) reported at a significantly higher rate compared with GW veterans without this history. These findings remained even after conservatively adjusting for multiple comparisons. Five of the 8 health symptoms correspond to 1 of the 3 categories (ie, fatigue, musculoskeletal, and mood-cognition) that define CMI criteria. Specifically, the symptom "fatigue or easily tired" is consistent with the CMI category of fatigue. The remaining 4 symptoms are consistent with CMI categories of musculoskeletal (ie, muscle weakness or fatigue, neck aches or stiffness, and backaches) and mood-cognition (ie, excessive anger or irritability).

Taken together, these findings suggest that TBI in this cohort of GW veterans is associated with specific health symptoms, several of which likely contribute to CMI diagnosis. This accords with our additional observation that a history of self-reported TBI in this veteran population was associated with an increased rate of CMI. Previously, CMI has been primarily attributed to certain war-related environmental exposures, such as sarin/cyclosarin, pesticides, and the use of anti-nerve gas pills.<sup>15,22</sup> In addition to these exposures, results from this study indicate that TBI may also contribute to CMI.

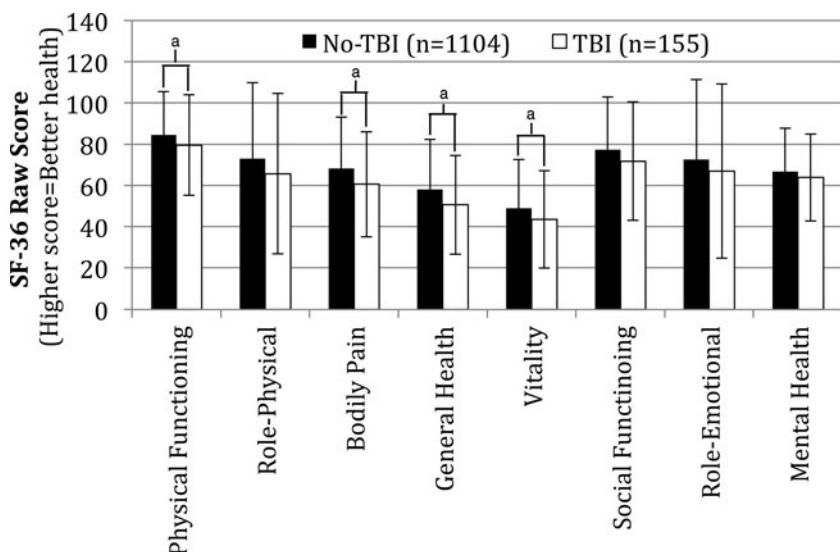


**Figure 2.** Mean scores for the Physical and Mental Health summary scores of the SF-36. Error bars represent standard deviation. The TBI group reported significantly more problems on Physical Health, but not Mental Health. <sup>a</sup> $P < .05$ . TBI, traumatic brain injury; SF-36, RAND 36-Item Health short Form Survey.

This is particularly relevant, as multiple brain insults (eg, pesticides and TBI) may cause an abnormally persistent neuroimmune response with each subsequent insult, contributing to chronic health symptoms such as those seen in GW veterans.<sup>45-48</sup>

Gulf War veterans with self-reported TBI also reported worse health-related quality of life associated with physical but not mental health as indicated by scores on the SF-36. Evidence suggests that clinically or socially mean-

ingful differences in MCS and PCS scores range from 2.5 and 5 points.<sup>49-51</sup> Gulf War veterans with a self-reported history of TBI endorsed average scores approximately 3 points lower than veterans without this history on the PCS, indicating a clinically meaningful difference in physical functioning. This finding adds to the existing literature in other populations such as OEF/OIF veterans, indicating that TBI contributes to a wide range of health symptoms and poorer quality of life.<sup>16,52</sup> Gulf



**Figure 3.** Mean score for each of the 8 individual subscales for the SF-36. Error bars represent standard deviation. The TBI group reported significantly more problems on the following individual subscales: Physical Functioning, Bodily Pain, General Health, and Vitality. <sup>a</sup> $P < .0125$ . TBI, traumatic brain injury; SF-36, RAND 36-Item Health short Form Survey.

War veterans and OEF/OIF veterans report some similar health symptoms, which may stem, in part, from exposure to TBI.<sup>53</sup> Gulf War veterans initially reported health concerns immediately upon returning from war, and, in general, these symptoms have persisted or worsened over the past 3 decades.<sup>13,14,54,55</sup> The long-term trajectory of the health and functioning of OEF/OIF veterans is unknown. However, if TBI is contributing to the health symptoms reported by both veteran groups, then OEF/OIF veterans may also experience persistent health outcomes similar to those of GW veterans. There is already some evidence indicating that OEF/OIF veterans are developing chronic long-term debilitating health problems. For example, a cohort of these veterans reported a slowly progressive worsening of health-related quality of life, as indicated by scores on the same measure used in this study (ie, SF-36).<sup>52</sup> Factors contributing to these problems were not explored, but Falvo and colleagues<sup>52</sup> suggest that mTBI and respiratory-related illnesses may be contributing to these findings. Change in self-reported quality of life is particularly worrisome, as it is associated with increased healthcare utilization and mortality in veterans.<sup>56-58</sup> However, it is important to highlight that the present findings may or may not apply to OEF/OIF veterans due to differences in TBI assessment. In general, studies of OEF/OIF veterans have used external verification of TBI, whereas this study relied on self-report, which is more likely to be influenced by systematic response bias.

Although this study has its strengths, there are a number of limitations that warrant discussion. Retrospective self-report data were used with no external confirmation of TBI. Furthermore, this study only asked veterans to indicate the presence or absence of a TBI. No information was collected on the nature of the injury such as timing (ie, before, during, or after the war), severity, frequency, or cause. This information would be useful to collect in future studies to determine whether health outcomes vary depending on the nature of injury (eg, 1 TBI vs multiple TBIs, mild vs moderate). Self-report of TBI may be unreliable if veterans were unaware that they had a TBI. It is also possible that some veterans had a tendency to endorse items indiscriminately (ie, systematic response bias). Future studies would benefit from external verification of TBIs, such as clinical evaluations to increase accuracy of reporting. Use of an updated definition of TBI would also be beneficial to future studies in increasing accuracy of reporting, as

scientific understanding of TBI has changed over the past few years.<sup>59</sup> Studies with other populations have demonstrated significant increases in self-reports of TBI when a clear definition is provided.<sup>60</sup>

This study is also limited by the use of a single diagnostic criterion for defining the cluster of symptoms related to the 1990 to 1991 Gulf War. More than a half-dozen diagnostic criteria for this syndrome have been developed,<sup>61</sup> including recent recommendations by the Department of Defense and Department of Veterans Affairs.<sup>21</sup> This study used the most commonly cited diagnostic criteria,<sup>20</sup> but different outcomes may be found if other classification systems are used.

In addition, the findings may be confounded by higher rates of psychiatric conditions in veterans with TBI. Both TBI and psychiatric conditions can result in increased health symptoms and decreased quality of life,<sup>62-67</sup> though the temporal relationship appears to be bidirectional. In this study, participants indicated the presence or absence of "frequent anxiety or nervousness" or "frequently feeling depressed." The rates of self-reported anxiety and depression between veterans with and without a self-reported history of TBI did not reach statistical significance. Likewise, there were no significant differences between groups on the MCS from the SF-36 or the Mental Health subscale of the SF-36, the latter of which assesses anxiety and depression. Taken together, these findings suggest that psychological health is unlikely to explain the present findings, though comprehensive psychiatric evaluations were not performed.

To our knowledge, this is the first study to examine whether reported TBI is associated with health symptoms, CMI, and health-related quality of life in GW veterans. Our results indicate that those reporting a history of TBI compared with no TBI also report significantly more health symptoms, increased rate of CMI, and poorer health-related quality of life. Past research has focused on a wide range of causes for CMI such as environmental exposures and contaminants, which have also been the primary focus for treatment.<sup>15</sup> Further research is needed to determine whether history of TBI is a causative factor of longer-term CMI and related health symptoms, either alone or as part of a multiple-hit hypothesis. Potential research could include investigating the role of TBI in combination with the environmental exposures unique to GW deployment.

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## Article

# Multiple Mild Traumatic Brain Injuries Are Associated with Increased Rates of Health Symptoms and Gulf War Illness in a Cohort of 1990–1991 Gulf War Veterans

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**Abstract:** Recent research demonstrated a relation between traumatic brain injury (TBI), health symptoms and diagnosis of Gulf War Illness (GWI) in Gulf War Veterans, but no study has examined the impact of multiple mild TBIs (mTBIs). A total of 229 male Gulf War Veterans from the Ft Devens Cohort were categorized by a number of mTBIs reported. One-way ANOVA and chi-square test of independence were used to test for differences in total reported health symptoms and diagnosis of chronic multisymptom illness (CMI) or Kansas GWI criteria, two of the most common case definitions of GWI. A total of 72 veterans reported no mTBIs (31.4%), 26 reported one mTBI (11.4%), 25 reported two mTBIs (10.9%), and 106 veterans reported sustaining three or more mTBIs (46.3%). Veterans reporting two or more mTBIs ( $p < 0.01$ ) or three or more mTBIs ( $p < 0.001$ ) endorsed significantly higher rates of health symptoms than Veterans reporting no mTBIs. Significantly higher rates of CMI ( $p = 0.035$ ) and Kansas GWI criteria ( $p < 0.001$ ) were seen in the three or more mTBI group. Results suggest two mTBIs increase risk of health symptoms, but three mTBIs may be the threshold needed to sustain chronic symptom reporting needed for a formal diagnosis. These findings highlight the importance of implementing policies and procedures monitoring head injuries in military personnel.

**Keywords:** mild traumatic brain injury; Gulf War Illness (GWI); chronic multisymptom illness; Gulf War

## 1. Introduction

Veterans from the 1990–1991 Gulf War have consistently reported health symptoms since returning from the Middle East almost three decades ago [1–13]. Hallmark symptoms of the syndrome, commonly known as Gulf War Illness (GWI), include fatigue, musculoskeletal pain, respiratory problems, skin problems or rashes, gastrointestinal difficulties, changes in mood, and cognitive difficulties. Currently, etiological factors leading to GWI remain unclear, but common hypotheses include neurotoxicant exposures while in theatre, such as prophylactic treatments (e.g., pyridostigmine bromide pills) and environmental exposures (e.g., pesticides, sarin gas) [14–17]. The diverse range of symptoms and unclear etiological factors has made the diagnosis of GWI difficult. As a result,

multiple diagnostic criteria have been developed. Two of the most commonly used criteria include Chronic Multisymptom Illness (CMI) developed by the Centers for Disease Control and Prevention and the Kansas GWI criteria [18–20]. Recent research demonstrated a relation between traumatic brain injury (TBI), health symptoms and rates of CMI in GW Veterans, which had not been previously examined [21]. However, to our knowledge, no study has examined the impact of multiple mTBIs on health symptoms or diagnosis of GWI in Veterans from the 1990–1991 Gulf War.

TBIs and mild TBIs (mTBIs), are of increasing concern, especially in cohorts where individuals are more likely to sustain multiple head injuries, such as sports communities and Veteran populations [22]. Mild TBI, also known as a concussion, occurs when an impact to the head or body results in one or more of the following: loss of consciousness (LOC) less than 30 minutes, inability to remember events immediately before or after the injury, alteration of consciousness (e.g., dazed, confused, disoriented), post-traumatic amnesia less than 24 hours, or neurological deficits [23]. Over the last decade, researchers have put-forth an overwhelming amount of evidence demonstrating significant chronic negative effects of repetitive mTBIs in individuals participating in contact sports, such as football [24,25]. Simultaneously, research on the impact of mTBIs in military populations has increased due to the use of improvised explosive devices (IEDs) in Operation Enduring and Iraqi Freedom (OEF/OIF).

A post-deployment survey study of OEF/OIF Veterans found that approximately 17% reported sustaining a mTBI in theater, with 59% of those reporting more than one mTBI. Veterans reporting mTBIs were at a higher risk for experiencing health symptoms, including headaches, cognitive problems, chest pain and gastrointestinal problems. Furthermore, veterans reporting more than one mTBI had an increased risk of experiencing health symptoms, such as headaches and sleep disturbances, compared to those only reporting a single head injury [26]. Studies specifically addressing the effects of multiple head injuries in military populations have found higher rates of post-concussive symptoms and sleep disturbances in Soldiers reporting multiple TBIs [27,28].

Recent research from the Ft. Devens Cohort Study demonstrated an association between self-reported TBI, chronic health symptoms and rates of CMI in Gulf War Veterans [21]. Veterans, surveyed in 1997–1998, self-reporting a TBI were more likely to meet CMI criteria and reported higher rates of chronic health symptoms than those who did not report a TBI. However, the study was limited due to a lack of information on the number and severity of TBIs experienced. The study also only examined rates of CMI, which has broad diagnostic criteria. Alternatively, the Kansas GWI criteria are more stringent. Recently, the Institute of Medicine (IOM) concluded CMI criteria should be used in clinical evaluations and Kansas GWI criteria should be used in research studies [29]. Comprehensive research should include diagnosis based on both case definitions. Therefore, the purpose of the current study was to determine whether GW Veterans reporting mTBIs were also reporting higher rates of chronic health symptoms and were more likely to meet CMI and/or Kansas GWI criteria. It was hypothesized that Veterans reporting more mTBIs would be more likely to report higher rates of health symptoms and more likely to meet CMI and/or Kansas GWI criteria.

## 2. Materials and Methods

### 2.1. Participants

Participants included 229 male Gulf War Veterans from the Ft. Devens Cohort Study, which is composed of Veterans who returned from war in 1991 through Ft Devens, Massachusetts. Initial assessments collected in 1991 and 1992–1993 were designed to assess psychological re-adjustment post-deployment. Later assessments collected in 1997–1998 and 2012–2014 included physical and emotional health concerns [3,4,6,7,9,10]. Review board approvals were obtained from the appropriate institutions prior to initiating survey distribution. Cross-sectional data collected from the 2012–2014 survey were analyzed for this study.

## 2.2. Self-Report of TBI

The following description of mTBI was provided:

*"Some people have the misconception that mild traumatic brain injury (also known as 'concussion') only happens when you lose consciousness after being hit on the head or when the symptoms last for a long time. However, a mild traumatic brain injury occurs anytime you have an impact to the head that causes symptoms for any amount of time (e.g., seconds or longer). These symptoms include: sensitivity to light or noise, headache, dizziness, balance problems, nausea, vomiting, trouble sleeping, fatigue, confusion, difficulty remembering, difficulty concentrating, or loss of consciousness."*

Veterans were asked to self-report the number of head injuries sustained and total number of reported head injuries were summed and Veterans were categorized into one of four groups: no mTBIs, one mTBI, two mTBIs, and three or more mTBIs.

## 2.3. Health Symptom Checklist

The health symptom checklist is a 34-item self-report questionnaire assessing the presence and absence of bothersome health symptoms over the past 30 days. The symptoms spanned a range of body systems (e.g., cardiac, dermatological, gastrointestinal, genitourinary, musculoskeletal, neurological, neuropsychological, psychological, and pulmonary). Veterans were instructed to check "yes" if the symptom had been present or "no" if it had been absent, during the past 30 days. Number of self-reported health symptoms was summed for each Veteran.

## 2.4. Chronic Multisymptom Illness Criteria

CMI criteria, as defined by the Centers for Disease Control, include the presence of persistent health symptoms for at least 6 months in 2 of the following 3 categories: fatigue, musculoskeletal factors, and mood and/or cognition [18]. Veterans completed a questionnaire in which they self-reported the presence or absence of symptoms in each domain over the past 6 months. Based on their responses, Veterans were classified as meeting CMI criteria or not meeting CMI criteria. Six veterans, 2 in the no mTBI group, 2 in the one mTBI group, 1 in the two mTBI group, and 1 in the three mTBI group, did not have sufficient information to assess CMI status and were excluded from the analysis.

## 2.5. Kansas Gulf War Illness Criteria

Kansas GWI criteria include the presence of moderate to severe health symptoms that began during or after the Gulf War in 3 of the following 6 categories: fatigue, pain, neurological and/or cognitive and/or mood, skin, gastrointestinal, and respiratory [20]. Exclusion criteria include the diagnosis of a serious medical or psychiatric condition that could account for symptoms or influence accurate symptom reporting. Veterans completed a questionnaire in which they self-reported the presence and severity of symptoms, and indicated whether the symptom occurred before, during or after deployment and were classified accordingly as meeting Kansas GWI criteria or not.

## 2.6. Statistical Analysis

Due to the non-normal distribution of reported total number of mTBIs, a Spearman correlation was used to assess the relation between total number of self-reported health symptoms and total number of self-reported mTBIs. A one-way ANOVA was performed to test for a difference in mean health symptoms endorsed among the four mTBI groups. Post-hoc analyses utilized Tukey's Honest Significant Difference test to determine which specific mTBI groups were significantly different. The chi-square test of independence or Fisher's exact test if expected cell counts were less than 5 was performed to test for differences in CMI or Kansas GWI criteria rates. Standardized residuals adjusted for multiplicity using the Bonferroni method were used to determine which mTBI group was contributing to a significant result. While multiplicity was accounted for within each analysis,

it was not accounted for across each analysis. Therefore, an alpha level of 0.05 was adopted for each individual analysis. Due to the wide range of total number of mTBIs reported, additional sensitivity analyses were conducted restricting the upper range of mTBIs to the third quartile to ensure outliers were not overly influencing the results.

### 3. Results

#### 3.1. Participant Characteristics

Seventy-two veterans reported no mTBIs (31.4%), 26 reported one mTBI (11.4%), 25 reported two mTBIs (10.9%), and 106 veterans reported sustaining three or more mTBIs (46.3%). There were no significant differences between groups with respect to age, education and race (Table 1).

**Table 1.** Participant Characteristics by Mild Traumatic Brain Injury (mTBI) Group.

	No mTBI (N = 72)	One mTBI (N = 26)	Two mTBI (N = 25)	Three mTBI (N = 106)
Age, years	57.3 ± 9.0	57.2 ± 9.5	56.5 ± 7.7	54.4 ± 7.5
Education, years	14.5 ± 2.8	14.8 ± 2.7	13.8 ± 3.8	14.0 ± 2.7
% Caucasian	66 (91.7%)	26 (100.0%)	24 (96.0%)	95 (89.6%)

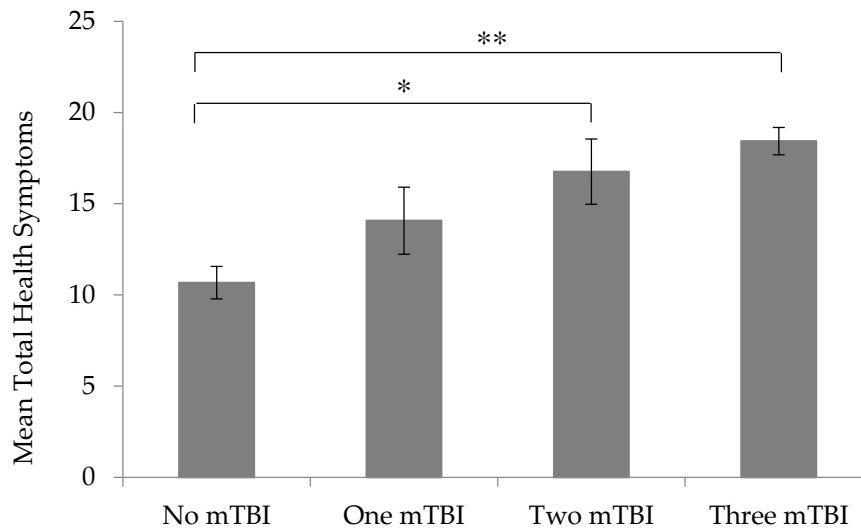
Note: No significant differences were seen between groups for participant characteristics.

#### 3.2. Correlation between Total Self-Reported Head Injuries and Health Symptoms

Overall, veterans reported a median of two mTBIs (IQR: 0–5) with a range 0 to 75 and a mean of 15.3 ( $sd = 8.7$ ) health symptoms. Total self-reported mTBIs was significantly positively correlated with total self-reported health symptoms ( $\rho = 0.417, p < 0.0001$ ), indicating Veterans reporting more mTBIs also reported more health symptoms.

#### 3.3. Health Symptom Checklist

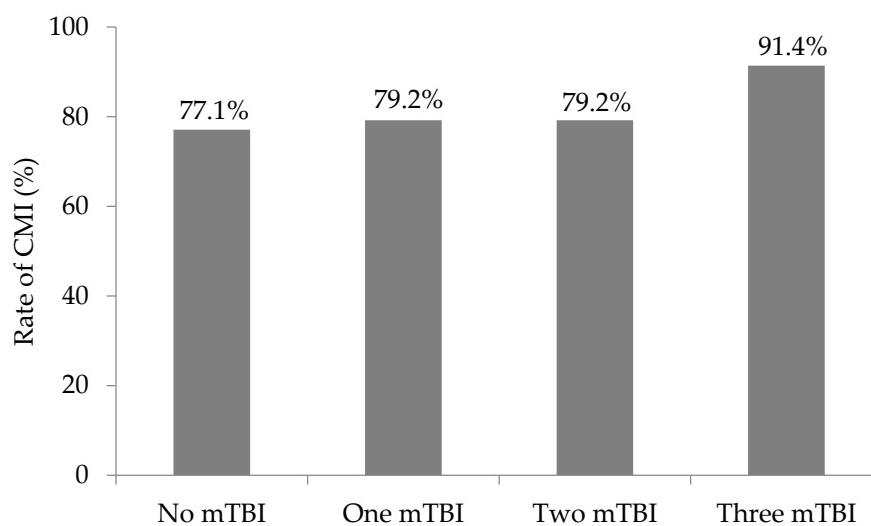
Veterans reported an average of 10.7 ( $sd = 7.5$ ) health symptoms in the no mTBI group, 14.1 ( $sd = 9.4$ ) health symptoms in the one mTBI group, 16.8 ( $sd = 9.0$ ) health symptoms in the two mTBI group, and 18.4 ( $sd = 7.7$ ) in the three or more mTBI group. The overall one-way ANOVA was significant ( $F = 13.93, p < 0.0001$ ) indicating at least one of the mTBI groups reported a significantly different number of health symptoms. Post-hoc analysis revealed that Veterans with two mTBIs reported significantly more health symptoms than Veterans reporting no mTBIs (mean difference = 6.1,  $p = 0.007$ ). Similarly, Veterans reporting three or more mTBIs reported significantly more health symptoms than Veterans reporting no mTBIs (mean difference = 7.8,  $p < 0.001$ ). No significant differences were seen between the remaining mTBI groups ( $p > 0.05$ ) (Figure 1). A sensitivity analysis restricting the range of mTBIs to 5 (which only affects the three or more mTBI group) still revealed a significant model ( $F = 7.55, p < 0.0001$ ). Veterans reporting three or more mTBIs still endorsed significantly more health symptoms than veterans not reporting a mTBI (mean difference = 6.3,  $p < 0.001$ ).



**Figure 1.** Mean total health symptoms by mild traumatic brain injury (mTBI) group. Error bars represent standard error. \*  $p = 0.007$ . \*\*  $p < 0.001$ .

### 3.4. Chronic Multisymptom Illness

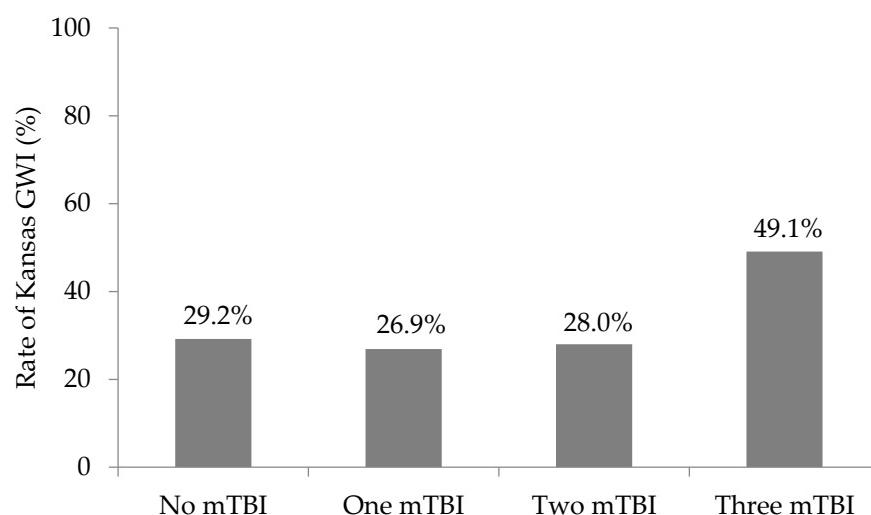
Overall, 84.3% of veterans met CMI criteria. Rate of CMI in the no mTBI group was 77.1% ( $n = 54$ ), 79.2% ( $n = 19$ ) in the one mTBI group, 79.2% ( $n = 19$ ) in the two mTBI group, and 91.4% ( $n = 96$ ) in the three or more mTBI group (Figure 2). Fisher's exact test revealed a significant difference between the mTBI groups ( $p = 0.035$ ). Adjusting for eight cells, the absolute value of a standardized residual greater than 2.7 ( $0.05/8 = 0.006$ , associated critical value of 2.7) indicates a particular cell is contributing to the significant result. The standardized residual for the three or more mTBI group meeting CMI criteria was 2.8 (−2.8 for the three or more mTBI group not meeting CMI criteria) indicating the rate of CMI in the three or more mTBI group was higher than expected. The standardized residuals for the remaining mTBI groups were less than 2.7. After restricting the range of mTBIs to 5 in a sensitivity analysis, 89.1% ( $n = 49$ ) of the veterans in the three or more mTBI group met criteria for CMI. However, the significant difference between the mTBI groups no longer remained ( $p > 0.05$ ).



**Figure 2.** Rate of Chronic Multisymptom Illness by mild traumatic brain injury (mTBI) group. Six veterans are missing CMI status (2 in the no mTBI group, 2 in the one mTBI group, 1 in the two mTBI group, 1 in the three mTBI group).  $p = 0.035$ .

### 3.5. Kansas Gulf War Illness

Overall, 38.0% of the veterans met Kansas GWI criteria. Rate of Kansas GWI criteria in the no mTBI group was 29.2% ( $n = 21$ ), 26.9% ( $n = 7$ ) in the one mTBI group, 28.0% ( $n = 7$ ) in the two mTBI group, and 49.1% ( $n = 52$ ) in the three or more mTBI group (Figure 3). The resulting chi-square test revealed a significant difference between the mTBI groups ( $p = 0.016$ ). Adjusting for eight cells, the absolute value of a standardized residual greater than 2.7 ( $0.05/8 = 0.006$ , associated critical value of 2.7) indicates a particular cell is contributing to the significant result. The standardized residual for the three or more mTBI group meeting Kansas GWI criteria was 3.2 (−3.2 for the three or more mTBI group not meeting Kansas GWI criteria). This suggests that more Veterans in the three mTBI group met Kansas GWI criteria than expected. The standardized residuals for the remaining mTBI groups were less than 2.7. After restricting mTBIs to a maximum of 5 in the sensitivity analyses, 51.8% ( $n = 29$ ) of the Veterans in the three or more mTBI group met Kansas GWI criteria. The chi-square test of independence still demonstrated a significant difference between mTBI groups ( $p = 0.027$ ). Similar to the main results, the standardized residuals for the three or more mTBI group meeting Kansas GWI criteria was 3.0 (−3.0 for the three or more mTBI group not meeting Kansas GWI criteria) indicating veterans were meeting criteria more than expected.



**Figure 3.** Rate of Kansas Gulf War Illness (GWI) criteria by mild traumatic brain injury (mTBI) group.  
 $p = 0.016$ .

## 4. Discussion

The current study examined the relation between the number of self-reported mTBIs and health symptoms in a cohort of 1990–1991 Gulf War Veterans. Though not previously considered to be a contributing factor, recent research revealed that Gulf War Veterans reporting a TBI also endorsed higher rates of health symptoms and were more likely to meet CMI criteria [21]. However, the study was limited due to a lack of information regarding number and severity of TBIs. The current study utilized follow-up data focusing exclusively on mTBIs in the same cohort of Gulf War Veterans. Overall, it was demonstrated that Gulf War Veterans reporting two or more mTBIs also endorsed higher rates of health symptoms compared to Veterans reporting no exposure to mTBIs. Additionally, Veterans in the three or more mTBI group meet both CMI and Kansas GWI criteria more than expected.

These results also coincide with research on multiple head injuries in other cohorts, such as sports communities, where individuals are at an increased risk for multiple mTBIs [22,24,25]. Within the last decade, research on repetitive mTBIs in football players has consistently demonstrated an association between multiple head injuries and chronic negative health effects. A prospective cohort study of collegiate football players across the United States found an association between repetitive concussions

and increased symptom duration and slower recovery time [30]. Studies of retired professional football players have consistently associated head injuries with worse health, increased rates of depression and an increased risk for neurodegenerative disorders years after play [31–33].

Negative consequences of multiple mTBIs are not exclusively limited to high-risk cohorts, but are also generalizable to the other populations. Increased rates of health symptoms were found in a community sample of adults and children sustaining a recurrent TBI of any severity within one year of an initial head injury compared to matched controls with no recurrent TBI [34]. The Transforming Research and Clinical Knowledge study collected information on individuals requiring a computed tomography scan after sustaining a head injury. Investigators found that individuals with a history of at least one head injury were more likely to report hepatic, musculoskeletal, spinal, neurological, pulmonary, and ear, nose or throat conditions than individuals sustaining their first TBI. Individuals with a history of TBI also reported higher rates of anxiety, depression and sleep disorders. Six months post-injury, individuals with a TBI history had higher rates of somatic symptoms, depression, anxiety, and worse processing speed and verbal learning. Additionally, individuals with a history of TBI were less likely to have returned to work [35]. However, it should be noted that in this study only 82% of the index head injuries were mTBIs and history of head injury had to be accompanied by a loss of consciousness. Unsurprisingly, individuals with a history of TBI have been found to have lower life satisfaction one-year post-head injury compared to individuals with no such history [36].

Currently, the threshold for the number of mTBIs an individual can sustain before experiencing negative consequences is unclear. If a threshold exists, it could directly impact policy. The results of this study suggest that the threshold may be two mTBIs for chronic health symptoms, as Veterans in the two mTBI and in the three or more mTBI group endorsed significantly more health symptoms than Veterans with no mTBIs. This is further strengthened by the finding that Veterans endorsing one mTBI reported similar rates of health symptoms as veterans with no mTBIs.

Our health symptom findings coincide with Miller and colleagues [27] research, which demonstrated that active duty Soldiers with two or more head injuries (<3 months) reported more health symptoms. Alternatively, work from Dretsche and colleagues [28] in active duty Soldiers preparing for deployment found an association between three or more concussions and increased rates of post-concussive symptoms measured by the neurobehavioral symptom inventory, which coincides with our CMI and Kansas GWI criteria findings. However, Miller et al. [27] only grouped Soldiers by no, one, or two or more mTBIs. Therefore, it is possible that the Soldiers with three or more mTBIs were driving the higher rate of symptom endorsement, which would be more consistent with the work of Dretsche [28].

Differences between the current study and Dretsche et al. [28] may account for the different health symptoms findings. Dretsche [28] studied active duty Soldiers whose head injuries were most likely more recent than the current study, in which veterans most likely sustained their injuries when they were active duty over two decades ago. The average age of the active Soldiers in Dretsche et al. [28] 26 years old, was also much younger than the average age in the current study. The effects of multiple mTBIs on chronic health symptoms later in life may be different than the effects seen closer to time of injury.

Finally, Gulf War veterans are a unique population due to the unique neurotoxicant exposures they encountered while in theatre, including pesticides and nerve agents, which may compound the effect of head injuries. This is known as the multiple hit hypothesis, which suggests multiple insults to the nervous system can cause chronic neuroinflammation due to a persistent neuroimmune response [37–39].

Contrary to our health symptom results, our findings demonstrated that Veterans reporting three or more mTBIs were more likely to meet CMI or Kansas GWI criteria. The Kansas GWI criteria results were particularly striking, as the rate of diagnosis nearly doubled in the group of Veterans reporting at least three mTBIs compared to Veterans not reporting any mTBIs. However, Veterans endorsing two mTBIs did not endorse higher rates of CMI or Kansas GWI criteria. It is important to note that the CMI results were no longer significant in the sensitivity analysis when the number of mTBIs were reduced to 5. This may suggest that the initial result may have been influenced by a limited number of Veterans reporting a high number of mTBIs. However, the difference in the sensitivity analysis between the

CMI and Kansas GWI illness may be a reflection of the diagnostic criteria themselves. CMI criteria are broad and may potentially over diagnose Veterans, as demonstrated by the high rate in this sample (84.3%). Kansas GWI criteria are more stringent (38.0% in this sample), but may exclude some Veterans with other comorbid conditions. While the health symptom results and the diagnostic criteria results in this study are seemingly contradictory at first, the difference in the results may suggest that while it may only take two mTBIs to increase rates of health symptoms, it may take at least three mTBIs before symptoms are severe enough for a Veteran to meet criteria for a formal diagnosis. However, this result needs to be interpreted with caution as more research is needed, especially regarding the timing of mTBIs in relation to symptom development, and other exposures which may contribute to (GWI).

While the current study has its strengths, there are some limitations inherent in the study design that should be addressed. Though veterans were provided with a mTBI definition that has been used in a multitude of published studies, only retrospective self-report data was used with no external verification of mTBIs. This may be particularly relevant in the current study, as Veterans are asked about events occurring over two decades prior. Timing of the mTBIs, whether they occurred before, during or after the war, was also not assessed. Further, if Veterans reported multiple mTBIs, the time between each mTBI was not collected. Both of these factors may alter the effect on health symptoms, and should be assessed in future studies. Similarly, health symptoms were self-reported with no clinician involvement. It is possible that some Veterans tended to endorse items indiscriminately or Veterans reporting more mTBIs were primed to report more health symptoms (e.g., response bias). Clinical evaluations for both mTBI exposure and symptom reporting may be beneficial to future studies.

To our knowledge, this is the first study to examine the association between multiple mTBIs, health symptoms and rate of CMI or Kansas GWI criteria in a cohort of Gulf War Veterans. Both subjective and objective evidence continues to support the seriousness of repetitive brain injuries in multiple diverse cohorts. However, the threshold for the number of head injuries that can be sustained before an increase in risk of chronic symptoms still remains unclear. The current study indicates that as few as two mTBIs may significantly increase the risk of chronic health symptoms, but three mTBIs may be the threshold needed to sustain chronic symptom reporting. These findings highlight the importance of implementing procedures and policies to closely monitor head injuries within the military.

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**Conflicts of Interest:** The authors declare no conflict of interest. The opinions or assertions contained herein are the private views of the author(s) and are not to be construed as official or as reflecting the views of the Department of Veterans Affairs, Army or the Department of Defense.

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